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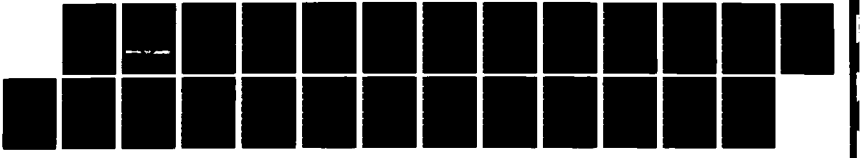

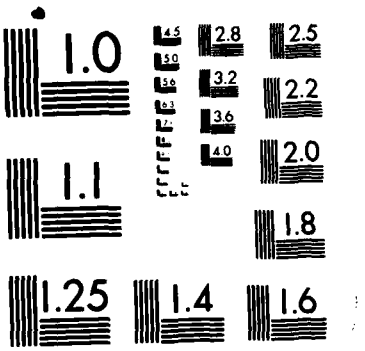

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REPORT NO. T7/84

ALBUMIN AFFECTS HYDRAULIC CONDUCTIVITY AND PERMEABILITY IN HOLLOW FIBER BUNDLES

US ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE
Natick, Massachusetts

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This study was conducted as a characterization of an artificial tubular surface to be used as a substrate for endothelial cell culture. Pressure-flow relationships, hydraulic conductivities (CFC) and permeability (p . S) to dextrans (3k, 40k, 70k) were determined for these hollow fiber bundles. Flow was a linear function of applied pressure. Although these tubes offer some resistance to flow, further resistance was a direct function of albumin concentration in the perfusate. CFC's ranged from 1-100 (ml/sec/cm^2/cm H_2O) x 10^8 and

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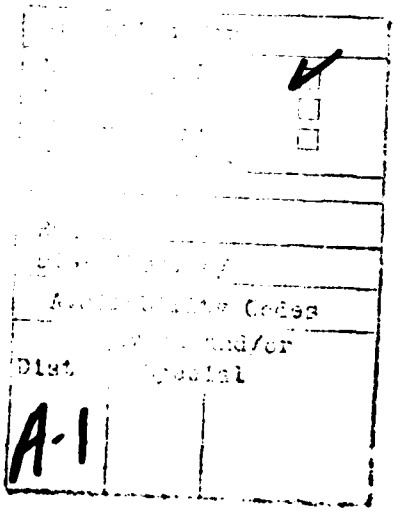
decreased in a predictably linear fashion with increasing perfusate albumin content. Reabsorption of filtered fluid also was highly correlated with perfusate albumin content. Finally, this hollow fiber bundle indicated a graded restriction to the permeability of dextran tracers, when perfusate albumin content was raised to 1%, and the effect of albumin on the permeability of these capillaries to dextrans was strongest for the highest molecular weight species. The effect of albumin on CFC appears to be a function of oncotic pressure. This conclusion is strengthened by data on the reabsorption of filtered fluid. Furthermore, the attenuation of 40k and 70k dextran permeability suggests a role for albumin in the regulation of macromolecular leakage from this microvascular model and this may involve an albumin-matrix or albumin-dextran interaction.



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NO. T 7/84

ALBUMIN AFFECTS HYDRAULIC CONDUCTIVITY AND PERMEABILITY IN HOLLOW FIBER BUNDLES

by

Stephen P. Bruttig and Ana L. Rodriguez

Project Reference: March 1984 Series:CR

US ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE

Natick, MA 01760

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ABSTRACT

This study was conducted as a characterization of an artificial tubular surface to be used as a substrate for endothelial cell culture. Pressure-flow relationships, hydraulic conductivities (CFC) and permeability (P • S) to dextrans (3k, 40k, 70k) were determined for these hollow fiber bundles. Flow was a linear function of applied pressure. Although these tubes offer some resistance to flow, further resistance was a direct function of albumin concentration in the perfusate. CFC's ranged from 1-100 (ml/sec/cm^2/cm H_2O) x 10^8 and decreased in a predictably linear fashion with increasing perfusate albumin content. Reabsorption of filtered fluid also was highly correlated with perfusate albumin content. Finally, this hollow fiber bundle indicated a graded restriction to the permeability of dextran tracers, when perfusate albumin content was raised to 1%, and the effect of albumin on the permeability of these capillaries to dextrans was strongest for the highest molecular weight species. The effect of albumin on CFC appears to be a function of oncotic pressure. This conclusion is strengthened by data on the reabsorption of filtered fluid. Furthermore, the attenuation of 40k and 70k dextran permeability suggests a role for albumin in the regulation of macromolecular leakage from this microvascular model and this may involve an albumin-matrix or albumin-dextran interaction.

keywords: Artificial Capillary

INTRODUCTION

Most often, the study of cultured vascular endothelial cells has been accomplished by investigation of the behavior of monolayer cultures in petri dishes. Studies are now beginning to emerge utilizing microcarrier bead technology (Ryan et al., 1980; Pearson, 1983), but to date, no one is using an artificial microtubular network of endothelium to study vascular/microvascular phenomena. As we wished to do just that (i.e., culture endothelial cells in an artificial microvascular network), we chose as our substrate the hollow polysulfone fiber bundle. However, there is little information available which would allow us to know what portion of function (flow, resistance, filtration, reabsorption, permeability) in such a system is due to the endothelium and what portion is an intrinsic property of the physical system proposed as a substrate. This information, especially hydraulic conductivity (CFC; Landis and Pappenheimer, 1963), is important, as we intend to use CFC to determine confluence of the cultured cells.

Microvascular filtration, reabsorption and permeability are normally events which involve the endothelial lining. In an artificial culture system, one should be able to study these events in isolation, yet interpret the results in light of the properties intrinsic to the acellular fiber bundle. Therefore, this study was undertaken with the major objectives of describing pressure flow and resistance features, as well as the hydraulic conductivity, reabsorptive and permeability characteristics of the proposed artificial attachment substrate.

Hydraulic conductivity is presented as a capillary filtration coefficient = CFC; $(ml/sec/cm^2/cm\ H_2O) \times 10^8$. Reabsorption is presented as a capillary reabsorption coefficient (= CRC, with the same units as CFC). Permeability is assessed as the permeability • surface area product (P • S; Renkin, 1964), where changes in this product are expected to reflect only changes in permeability,

since surface area is constant. Finally, resistance is presented as 1- ($\dot{Q}o/\dot{Q}i$), where $\dot{Q}o$ is the observed flow rate and $\dot{Q}i$ is the ideal flow rate of 1 ml/min/mm Hg applied pressure (Dinnar, 1981).

MATERIALS AND METHODS

Hollow Microfiber (Capillary) Bundle. Commercially available Amicon polysulfone fiber bundles (Vitafiber Artificial Capillary Systems PS100; Amicon Corp., Lexington, MA) were used throughout this study (Fig. 1). These bundles contain 150 hollow fibers, each with an internal diameter of 200μ. The total available surface area was calculated to be 53.72 cm^2.

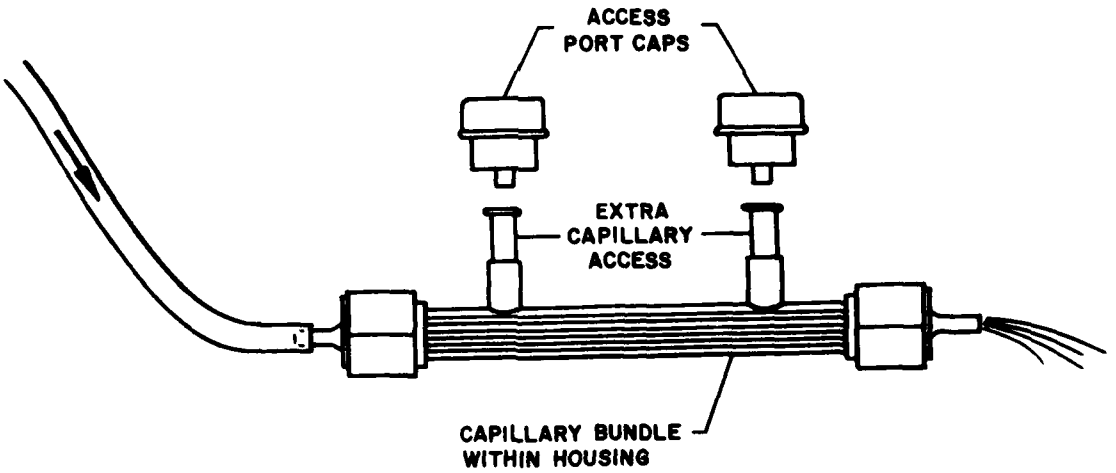

Figure 1. This figure is a schematic representation of the Vitafiber artificial capillary bundle. Inlet and outlet ports and access to extracapillary space are shown.

Hydraulic Conductivity (CFC) Measurements. Perfusate was applied to the fiber bundle as a hydrostatic column from a reservoir set at various heights above the capillary bundle. A 1 ml glass pipette (graduated in 1/100 ml) was led from a port which provided access to the "extra-capillary" space. Flow through the capillary bundle was measured and collected. Likewise, the rate of accumulation of filtered fluid was measured and a sample collected into pre-weighed vials for volume determination. Finally, a sample from the perfusate reservoir was collected after each run.

Capillary Reabsorption Coefficients (CRC). In order to determine reabsorption coefficients, filtration was allowed to occur as described for CFC above. Flow was stopped and the filtered volume (in the pipette) noted and then reabsorption was monitored for a measured time period. Reabsorption coefficients were then calculated as the volume (ml) of fluid reabsorbed per unit time (sec), per unit surface area (cm^2), per unit hydrostatic pressure (cm H_2O), in the same manner as for CFC.

Permeability Studies. Fluoresceinated dextrans (3,000, 40,000, 70,000 daltons; Sigma Chemical Co., St. Louis, MO.) were used as tracers in the capillary permeability determinations. These tracers were dissolved (individually) in phosphate buffer (PBS, 1 - 1.5 mg/ml). Perfusate protein content was adjusted (0 - 6g%) with bovine serum albumin (BSA; Fraction V Bovine Plasma, Armour Pharmaceutical Co., Kankakee, IL). Perfusate and filtrate samples were collected (cf. above) and the fluoresceinated dextran tracers were quantitated with a scanning spectrofluorometer (American Instrument Co., Silver Spring, MD) at an excitation wavelength (λ ex) of 480 nm and an emission wavelength (λ em) of 510 nm.

RESULTS

Pressure-Flow and Resistance Characteristics. The pressure-flow relationship was linear, as it has been described for other rigid tubular systems (Fig. 2). The parallel arrangement of the hollow fibers offers some resistance to flow and this resistance increased predictably with increasing amounts of albumin in the perfusate (Fig. 3). The increases in resistance (with increasing BSA) were interpreted to reflect the changes in the viscosity of the solution, as all other system parameters remained constant.

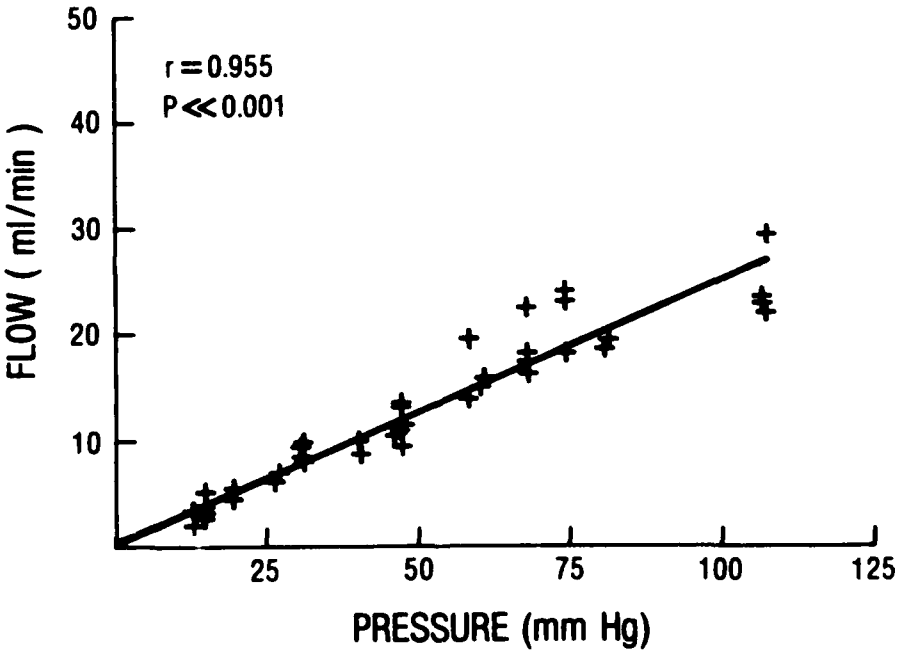

Figure 2. The relationship between pressure and flow in the hollow fiber bundle is depicted. Perfusate albumin concentration was 0 g/dl. Y = 0.386 + 0.250 (X).

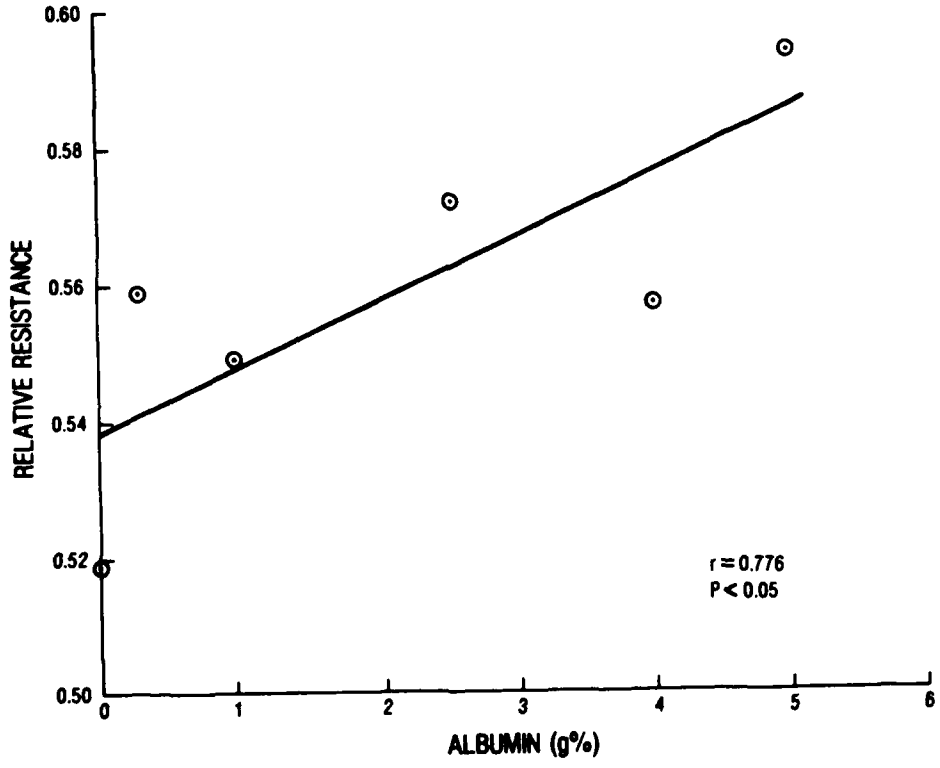

Figure 3. The resistance of the acellular hollow fiber bundle is plotted as a function of perfusate albumin concentrations (g/dl). Y = 0.5380 + 0.0094 (X). Points are means of individual observations.

Hydraulic Conductivity (CFC) Characteristics. The filtration of fluid through the capillary bundle was essentially independent of perfusion pressure ($r = 0.209$, $t = 1.847$, $n = 77$, $P > 0.05$). Between 10 and 70 cm H_2O, CFC ranged from 40 to 90 (ml/sec/cm^2/cm H_2O) x 10^8 . As the albumin concentration of the perfusate was increased, CFC fell progressively (Fig. 4) and CFC's = 0 were predicted for perfusate albumin contents of 6.04 g/dl. Finally, at the higher albumin concentrations (4 or 5 g/dl), we observed transient reabsorption of the filtered fluid. This "reabsorptive transient" was coincident with the cessation of flow through the system. We interpreted this result, that is the transient reabsorption of fluid by these capillaries, as reflecting the changes (increases) in oncotic pressure of the perfusate. Therefore, we conducted experiments to characterize the reabsorption of fluid by these capillaries.

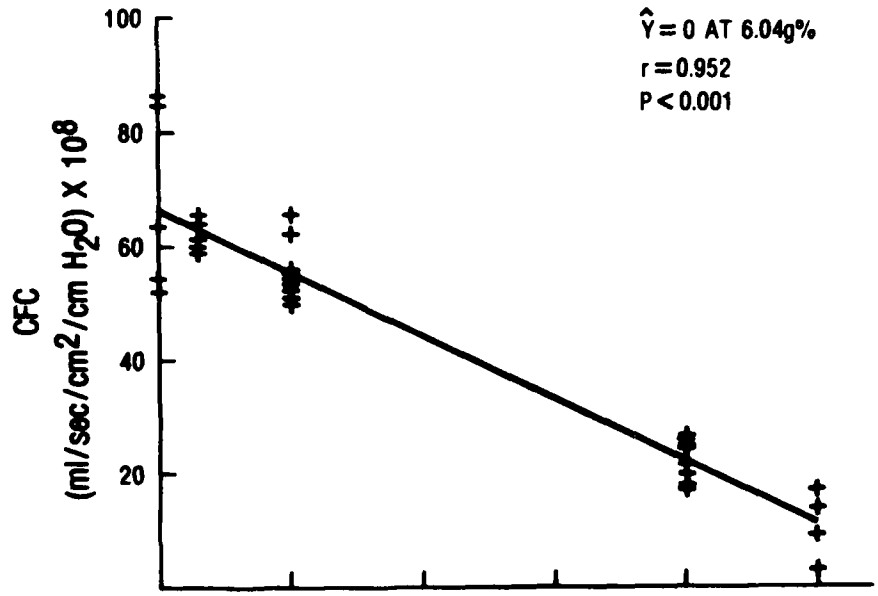

Figure 4. The relationship between capillary filtration coefficient and perfusate albumin content is depicted here. Y = 65.960 - 10.923 (X).

Reabsorption (CRC) Characteristics. The reabsorption of filtered fluid was in direct proportion to the perfusate albumin concentration. When CRC was expressed as a function of oncotic pressure (mm Hg), the equation of best fit was a power curve (Fig. 5). Although this equation predicts CRC = 0 when oncotic pressure = 0 mm Hg, we were unable to reliably measure CRC at oncotic pressures less than 10 mm Hg. In any case, the predictability of CRC, within the range of oncotic pressures normally encountered physiologically, was quite good.

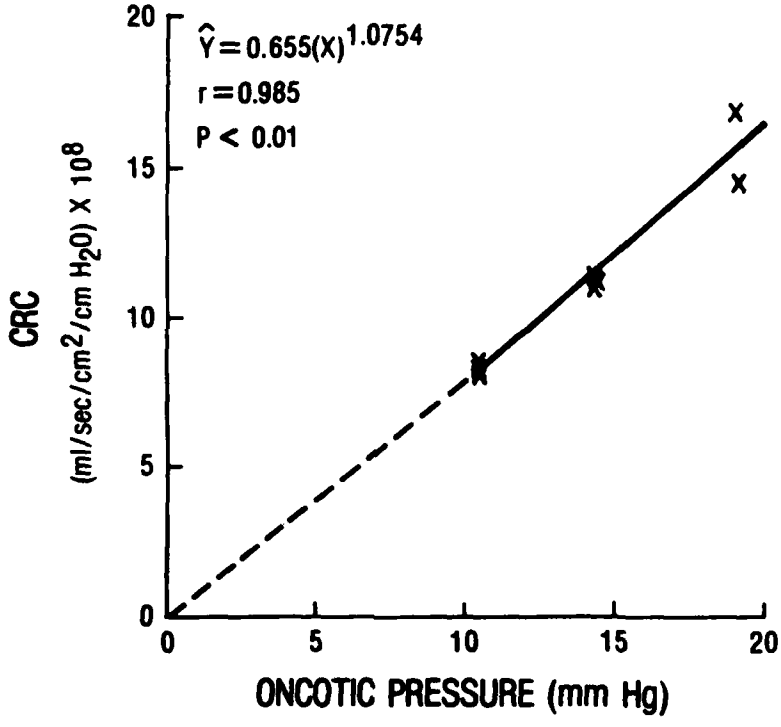

Figure 5. The relationship between capillary reabsorption coefficient (CRC) and oncotic pressure is plotted. Oncotic pressure was calculated from protein concentration as the average of values calculated from references Navar and Navar (1977) and Katz (1982).

Permeability of the Capillary Bundle to Dextrans. The Vitafiber Capillary Systems are manufactured to specific tolerances for pore size. The PS100 system used in these studies has a nominal cut-off of 100,000 daltons. As such, the capillary bundle was permeable to all of the dextran tracers used in the study. When permeability, expressed as the P • S product, is standardized for differences in fluid filtration (Curry, 1979; 1980), and the values are plotted versus the molecular weight in daltons, a logarithmic decay function obtains (Fig. 6). Interestingly, the overall character of this relationship is not unlike that observed in intact vascular beds (Grotte, 1956; Renkin, 1964; Renkin and Garlick, 1970). This graded restriction to the permeability of dextran tracers was not observed when the perfusate albumin content was below 1 g/dl.

The graded restriction to permeability observed in this study is believed to be a function of some albumin interaction either with the dextran tracer, the capillary matrix or both. This belief comes not only from the above-described graded permeability, but from the fact that for the larger tracers (40,000, and 70,000 daltons), permeability was negatively correlated with perfusate albumin content (Fig. 7). In addition, the strength of this relationship (P • S/CFC vs albumin content) increased in a highly predictable manner with increasing molecular size (Fig. 8).

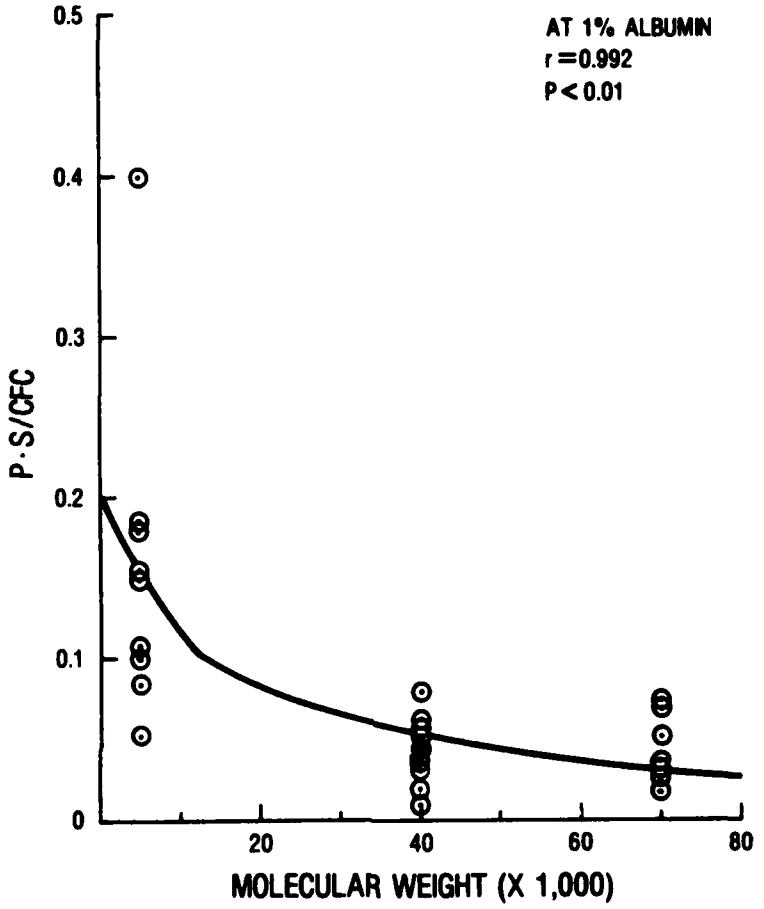

Figure 6. The permeability • surface area product, standardized for CFC, is plotted vs. molecular weight of the tracer molecule. Y = 0.199 - 0.039 ln X).

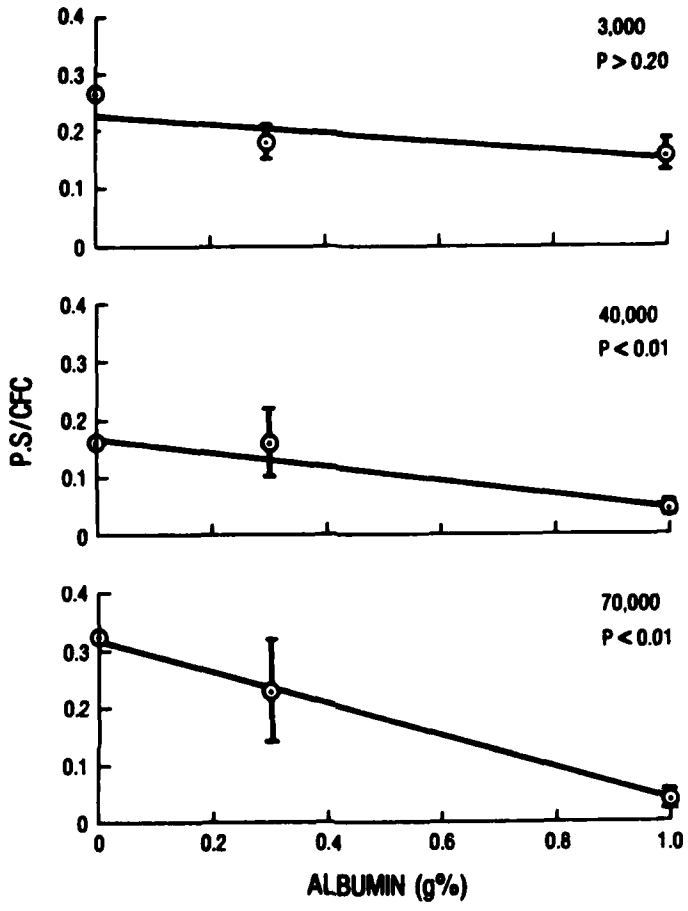

Figure 7. The relationship between P • S product, standardized for CFC and perfusate albumin content, is plotted. The upper panel shows the relationship for 3,000 dalton dextran molecules; the middle panel shows the relationship for 40,000 dalton dextran molecules; the lower panel shows the relationship for the 70,000 dalton dextran molecules.

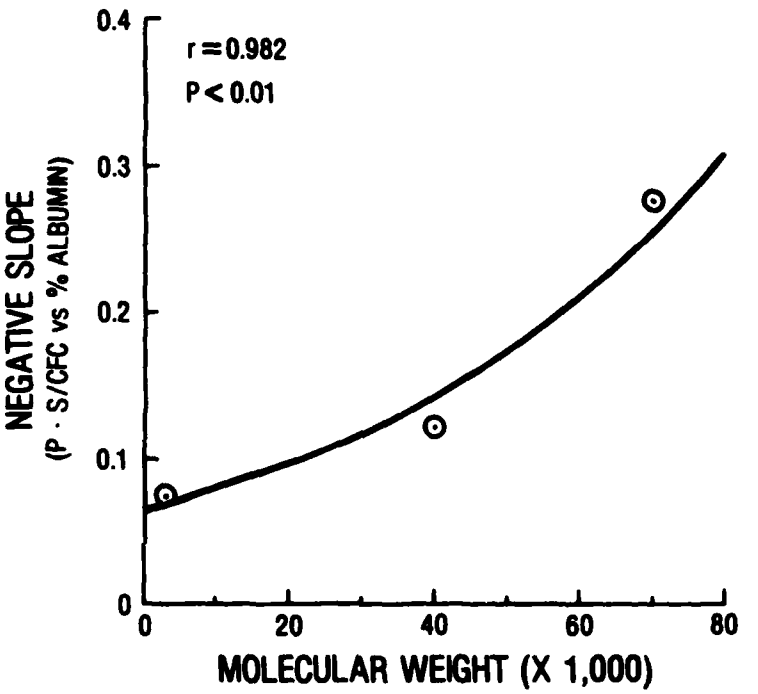

Figure 8. The slope of each plot in Figure 7 is plotted versus the molecular weight of the tracer described in that plot. This figure describes the "strength" of effect of albumin in reducing the permeability of larger and larger molecules. $Y = (0.0656e)^{0.0193\,(X)}$.

DISCUSSION

The use of the Vitafiber Artificial Capillary System for the culture of cells was described by Knazik et al. (1972). They grew tumor cells in the "extra-capillary" space in an effort to observe tumor tissue growth when fed in a physiologic manner (i.e., by filtration, diffusion, etc.) from a capillary network. We anticipate the use of this capillary bundle whose luminal surface will serve as an attachment substrate for cultured endothelial cells. By such methods, we will be able to study endothelial function in relation to flow and pressure changes (both constant and pulsatile) and in response to the shear forces normally encountered in vessels of this size. However, studies of this type require a confluent endothelial monolayer for observations such as filtration or permeability to have any validity. To provide a reasonable, non-invasive assessment of confluence, we chose the filtration of fluid from luminal to extra-capillary space as our primary indicator. As the monolayer covers the luminal surface, more and more of the fixed pores in the capillary wall will be covered by attaching cells. Thus, daily observations should indicate a fall in CFC values with growing cultures, and a plateau when the culture reaches confluence (as has been shown for endothelal cell growth on flat membranes). In order to utilize data like these effectively however, complete characterization of the acellular capillary system was necessary. The pressure-flow and resistance determinations made in the acellular capillary bundle are typical for rigid tubular systems. Changes in the resistance of the capillary system can be attributed only to the addition of protein, specifically albumin. Finally, since all other flow parameters were held constant, at times when albumin concentration was increasing, these observations on flow and resistance appear to be due mainly to albumin-induced changes in viscosity.

The hydraulic conductivity of the acellular capillary tubular membrane is within the range of values determined for some mammalian vascular beds (Landis and Pappenheimer, 1963). The specific changes in hydraulic conductivity (CFC) in the present of albumin might have been predicted in light of recent investigations by Curry and associates (Mason et al., 1977; Curry, 1979, 1980) by Michel and Phillips (1979) and by Turner et al. (1983). These investigators, using a frog mesentary, studied the effects of albumin on CFC. Their results, similar to ours, showed substantial decreases in CFC with increasing albumin content in the perfusate. However, on the basis of further experimentation, namely the capillary reabsorption coefficients (CRC), we would conclude that the effect of albumin on filtration in the present system, as well as reabsorption, is primarily one mediated by oncotic pressure. In addition, we feel that fluid flux through pores, whether the pores of an acellular artificial capillary membrane or the tortuous intercellular clefts of a vascular endothelial lining, may also be regulated to a limited extent by the viscosity of the solution which imparts some frictional resistance to flow. Our conclusions do not rule out the possibility that some of the decrease in CFC may be mediated by an albumin-matrix interaction (see Huxley and Curry, 1982), however, we would then expect to see a similar effect on CRC with increasing albumin concentration. In fact, we see the opposite. However, it must be remembered that the filtration surface of these experiments is a synthetic polysulfone matrix, and not the physiologically-derived endothelial cell-basement membrane complex of an actual capillary bed.

The observed permeability to dextrans was expected, given the pore size of the membrane. What was unexpected was the apparent effect of albumin solutions, i.e., a graded restriction to the permeability of variously-sized tracers. Nor did we expect to see an increasingly stronger restrictive effect with increasing molecular weight. The explanation of these phenomena is not at all

clear. It is known that albumin: 1) binds avidly to many surfaces; 2) imparts a certain viscosity to aqueous solutions; 3) increases the oncotic pressure of aqueous solutions; and 4) binds avidly to a variety of chemical species and/or drugs. For at least some of these reasons, one could expect that the observed results (which occurred in large measure because of the addition of albumin) may be explained as albumin-tracer, albumin-matrix and/or albumin-tracer-matrix interactions.

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